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Award Number: DAMD17-02-1-0660

TITLE: Role of Estrogen Metabolism in the Initiation of Prostate Cancer: Biomarkers of Susceptibility and Early Detection

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REPORT DATE: May 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-05-2005	Annual	1 May 2004 - 30 Apr 2 0 0 5

4. TITLE AND SUBTITLE
Role of Estrogen Metabolism in the Initiation of Prostate Cancer: Biomarkers of Susceptibility and Early Detection

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-02-1-0660

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Nebraska Medical Center
Omaha, NE 68198-6805

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
Treatment of Noble rats with testosterone plus estradiol (E2) induces prostate carcinomas. We think that estrogens initiate prostate cancer by reaction of catechol estrogen-3,4-quinone (CE-3,4-Q) metabolites with DNA. Formation of depurinating adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate prostate cancer. After treatment of rats with CE or CE-3,4-Q, CE metabolites and CE-glutathione (GSH) conjugates were lower in regions where tumors develop and methoxyCE were higher in regions where tumors do not develop. To study the role of CE-Q in initiation of prostate cancer, we are (1) treating rats with E2 and/or testosterone and analyzing the CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts in the regions of the prostate by HPLC with electrochemical and mass spectrometric detection; (2) studying in the prostate conversion of testosterone into E2 and its metabolism; and (3) determining the expression at the mRNA level of four selected enzymes involved in estrogen activation and deactivation in the prostate of rats treated with E2 and/or testosterone. These studies will provide information critical to understanding the molecular etiology of prostate cancer, identify biomarkers for early detection of susceptibility and lead to development of strategies for prostate cancer prevention.

15. SUBJECT TERMS
estrogens as endogenous tumor initiators; initiation of prostate cancer in Noble rats by estrogens; analysis of estrogen metabolites, conjugates and DNA adducts; expression of estrogen activating and deactivating enzymes

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	6	**19b. TELEPHONE NUMBER** *(include area code)*

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Introduction

The purpose of this research is to investigate the hypothesis that estradiol (E_2) initiates prostate carcinogenesis and testosterone promotes the process. This is being explored in male Noble rats, which develop prostate tumors when treated with E_2 and testosterone [1]. We think that estrogens are involved in the initiation of prostate cancer by a mechanism that involves oxidation of endogenous 4-catechol estrogen (CE) metabolites to CE-3,4-quinones (CE-3,4-Q). Reaction of CE-3,4-Q with DNA results in tumor initiation as the first step in the events leading to prostate cancer. Formation of depurinating DNA adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate the cancer [2]. To study the role of CE-Q in the initiation of prostate cancer, we are (1) treating male Noble rats with E_2 by i.p. injection at various doses and for various times, analyzing the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts and comparing their levels in the various regions of the prostate [3]; (2) investigating the conversion of testosterone into E_2 in the prostate by analyzing the same compounds in prostate tissues from rats treated with testosterone or testosterone plus the aromatase inhibitor letrozole; and (3) determining the expression of four enzymes involved in the activation and deactivation of estrogens, cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase (COMT) and quinone oxidoreductase (QOR). The results of these studies will provide information on the relationship between estrogen activation and deactivation in relation to tumor initiation in the prostate.

Body

In the third year of this research project, significant progress has been made on the projected tasks, as detailed in the Statement of Work, but some considerable hurdles have also been met. Based on these results, we have modified some of our proposed studies. The results of these studies are reported below.

Task 1: Conduct the E_2 dose-response study of CE metabolites, GSH conjugates and DNA adducts.

Previously, we treated Noble rats with 0, 16, 32 or 48 mg/kg of E_2 by i.p. injection, and after 3 h the prostate tissues were collected and analyzed by UNMC with electrochemical and mass spectrometric detection. As reported previously, very few metabolites or conjugates were detected at very low levels. One possible explanation of these results is that the treatment with E_2 needs to be sustained in order to find effects. To study this, we have initiated a study with treatment with E_2 by silastic implant. First, we determined the *in vitro* release from silastic implants of E_2 under physiologic conditions. The standard 1 cm-long implant we use released 1.5 Φg of E_2 per 24 h. This is more than 3 orders of magnitude below the lowest dose used in our previous experiments (~3-4 mg/rat). Thus, it is highly unlikely that we will be able to achieve a sufficiently high sustained E_2 dose without causing toxicity; the 1.5 Φg E_2 per 24 h dose already results in growth retardation, probably because of the strong estrogenic activity of this dose [1,4]. Simultaneous treatment with testosterone may be necessary for the detection of this type of E_2 effect. Therefore, we are planning a study in which rats will be treated with E_2 alone or in combination with implanted testosterone plus E_2 for 3 or 6 h, or implanted testosterone or vehicle alone.

On the basis of these results, we decided to change the approach and directly administer the catechol estrogens, which are not estrogenic and may be less toxic and more carcinogenic than the parent compound, E_2. Using an HPLC method to measure catechol estrogens in serum, which we developed, the *in vitro* release of 2-hydroxyE_2 (2-OHE_2) and 4-OHE_2 from silastic implants was measured under physiologic conditions. This release rate appeared to be at least 10-fold lower than that of E_2, indicating that the silastic implant approach is not feasible. Instead we decided to use the pellet method of Innovative Research, Inc. (Sarasoata, FL), which guarantees a sustained controlled release. We are in the process of determining the catechol estrogen dose in pellets needed to achieve an *in vivo* release rate similar to that of the silastic implants containing E_2. Once this dose has been identified, we will begin a cancer induction study with groups of Noble rats given 2-OHE_2 or 4-OHE_2 with and without additional

testosterone. The results will be compared with those from a group of rats given E_2 with testosterone, which is expected to develop a 100% prostate cancer incidence.

In a previous experiment, Noble rats were also treated with testosterone by silastic implants for 2 wk or by i.p. injection of 0 or 52 mg/kg for 6 h, and prostate tissues were collected for HPLC analyses with electrochemical and mass spectrometric detection. E_2 was detected in the prostate of rats injected with testosterone, but not in the control rats, indicating the presence of aromatase activity in the Noble rat prostate. To determinewhether inhibition of the aromatase enzyme would eliminate detectable E_2 in the prostate, we embarked on a study with the aromatase inhibitor Letrozole administered by silastic implant. However, it appeared that the physical properties of Letrozole make it almost impossible to prepare silastic implants. Thus, for this compound, we were also forced to turn to the pellet method of InnovativeResearch, Inc. (Sarasota, FL). We are in the process of testing the Letrozole dose required to inhibit the *in vivo* formation of E_2 from testosterone. Once this dose has been established, we will conduct a cancer induction study with groups of Noble rats given testosterone with and without addition of Letrozole.

Task 4: Analyze the expression of estrogen-metabolizing enzymes in control animals. We previously conducted analyses of the four enzymes CYP19, CYP1B1, COMT and NQO1 in prostate tissue from control rats at the mRNA level. This study was extended to protein expression determined by Western blot analysis for CYP19 and CYP1B1. Both enzymes were expressed at the protein level in the four areas of the prostate and the seminal vesicle. It is noteworthy that expression of both enzymes was higher in the dorsolateral prostate and urethra than in the other structures, including the ventral and anterior prostate. In the above summarized ongoing studies (Task 1), the expression of CYP19, CYP1B1, COMT and NQO1 will also be determined in the vehicle-treated groups.

Task 5: Begin analysis of the expression of estrogen-metabolizing enzymes in E_2-treated animals. We have previously analyzed expression of the four enzymes CYP19, CYP1B1, COMT and NQO1 at the mRNA level. We have extended this analysis to protein expression determined by Western blot for CYP19 and CYP1B1. E_2 treatment did not have a significant effect on the expression of either enzyme. Overall, the expression of these enzymes was reduced very slightly by E_2 treatment. In the above summarized ongoing studies (Task 1), the expression of the four enzymes CYP19, CYP1B1, COMT and NQO1 will also be determined in the hormone-implanted groups.

Key Research Accomplishments

1. Treatment with E_2 at sufficiently high doses to allow analysis for estrogen metabolites, estrogen conjugates and estrogen-DNA adducts has not been found to be feasible thus far.

2. Tissues from the E_2 and testosterone experiments were analyzed for expression of the estrogen-metabolizing enzymes CYP19 (aromatase), and CYP1B1 at the protein level. These enzymes are present at both the mRNA and protein levels, consistent with previous findings that suggested activity of these in enzymes in the Noble rat prostate [3].

Reportable Research Accomplishments

Singh, S., Bosland, M.C., Cavalieri, E.L. and Rogan, E.L. Effect of treatment with estradiol or testosterone on the expression of CYP19, CYP1B1, COMT and NQO1 in the prostate of male Noble rats. Manuscript in preparation..

Conclusions

In this third year, we have analyzed estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts in the regions of rat prostate after treatment with E_2 or testosterone. We have shown that following treatment with testosterone, the prostate contains significant amounts of E_2, which is not present in the prostates of untreated rats. We have determined the expression of four selected estrogen-metabolizing enzymes CYP19, CYP1B1, COMT and NQO1, in the regions of the prostate from control rats and rats treated with E_2 or testosterone. We have shown that all of these enzymes are, indeed, present in the rat prostate. Thus far, we have not detected estrogen-DNA adducts in the prostate tissue from rats treated with E_2, but other methodologies are coming available that may solve this problem for us. We have begun studies to show the effects of an aromatase inhibitor on the development of prostate tumors in rats treated with E_2 and testosterone. These studies will be completed in the following year.

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